Reversible cross-linking of alcohol and lactate dehydrogenases with the bifunctional reagent N2,N2'-adipodihydrazido-bis-(N6-carboxymethyl-NAD+*)

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Affinity precipitation with N2,N2'-adipodihydrazido-bis-(N6-carboxymethyl-NAD+) (bis-NAD+) as a means of isolating alcohol dehydrogenase (EC 1.1.1.1) and lactate dehydrogenase (EC 1.1.1.27) has already been reported (Flygare et al., 1983; Beattie et al., 1984). However, affinity precipitation of alcohol dehydrogenase in the presence of bis-NAD* and pyrazole has been found to give mixed results. The yeast enzyme requires a high ionic strength for precipitation, whereas the liver enzyme does not precipitate reproducibly. On the other hand, lactate dehydrogenase readily precipitates in the presence of bis-NAD* and oxalate, and this technique has been used with success to purify the enzyme from crude extracts (Flygare et al., 1983; Beattie et al., 1984). This communication reports the use of reversible cross-linking of horse liver alcohol dehydrogenase with bis-NAD* and pyrazole in conjunction with gel filtration, and also on the possible separation of the isoenzymes of lactate dehydrogenase by affinity precipitation.

The materials and procedures used in this communication were as described by Beattie et al. (1984); enzyme preparations were obtained from Sigma (Poole). Alcohol dehydrogenase from horse liver, a dimeric enzyme, was gel filtered through a column of Sephadex G-100 which had been equilibrated with 10 mm-pyrazole. Fig. 1 shows the elution profile of a sample of untreated enzyme with that of a sample that had been mixed with 2-NAD* equivalents of bis-NAD* per enzyme subunit before application to the column. The alcohol dehydrogenase that had been complexed with bis-NAD* eluted earlier than the uncomplexed enzyme. However, no alcohol dehydrogenase activity was detected in the void volume, indicating that polymeric aggregates were not formed. It is likely that the complexed enzyme is a dimer of dimers, as suggested by Flygare et al. (1983) from a study of complex formation between horse liver alcohol dehydrogenase, bis-NAD* and isobutyramide. The combination of these two techniques of reversible cross-linking with bis-NAD* and gel filtration is a promising alternative purification procedure where affinity precipitation is difficult to achieve.

Another application of affinity precipitation is the possible separation of isoenzymes of lactate dehydrogenase on the basis of abortive complex formation. The heart-type isoenzyme, whereas the liver enzyme does not do this to any significant extent. In a model study, isoenzymes (from pig heart and muscle; 23.6 μM of enzyme subunits) were affinity precipitated with bis-NAD* (1 NAD* equivalent per enzyme subunit) in the presence of saturating concentrations of either oxalate or the pyruvate analogue oxamate. Both isoenzymes were strongly precipitated when oxalate was used (70-90%). However, there was a striking difference in precipitation when oxamate was used. In the presence of oxamate, bis-NAD* gave 67% precipitation of the H, isoenzyme, whereas there was little or no precipitation of M, (<2%). This clear-cut difference in affinity precipitation between the two isoenzymes, based on differential abortive complex formation, is in agreement with an earlier affinity chromatographic study of O’Carra et al. (1974). Further studies on the application of affinity precipitation in isolating lactate dehydrogenase isoenzymes from crude extracts are in progress.

We are grateful to the National Board of Science and Technology (EOLAS) for a grant in support of this work.


Abbreviation used: bis-NAD*, N2,N2'-adipodihydrazido-bis-(N6-carboxymethyl-NAD+).

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Received 30 September 1988

Fig. 1. Gel-filtration of horse liver alcohol dehydrogenase samples on Sephadex G-100 equilibrated with pyrazole in the presence and absence of bis-NAD*.

A column of Sephadex G-100 (1 cm × 50 cm) was equilibrated with 20 mm-potassium phosphate buffer, pH 7.4, containing 10 mm-pyrazole. A sample of the enzyme was applied and eluted with the same buffer mixture (○). In a second experiment (●), the enzyme was mixed with bis-NAD* (2 NAD* equivalents/enzyme subunit) before gel filtration. The void volume of the column (V0) was determined by the elution of Dextran Blue 2000. Protein concentration was monitored by the absorbance at 280 nm.